# Glucocorticoid Receptor Expression Profiles in Mononuclear Leukocytes of Periparturient Holstein Cows

**M. T. Preisler,* P.S.D. Weber,* R. J. Tempelman,***
**R. J. Erskine,† H. Hunt,§ and J. L. Burton***
*Department of Animal Science and
†Department of Large Animal Clinical Sciences
Michigan State University, East Lansing 48824
§Avian Diseases and Oncology Laboratory
USDA-ARS, East Lansing 48823

## ABSTRACT

Cortisol-activated glucocorticoid receptors modulate cellular responses to stress by translocating from the cytosol to the nucleus and enhancing or repressing the transcription of target genes. The functional capacity of mononuclear leukocytes is inhibited in parturient dairy cows at a time when blood cortisol concentrations are high. Because the glucocorticoid receptor is autoregulatory in many cell types, the hypothesis of the current study was that glucocorticoid receptor expression by mononuclear leukocytes is altered around parturition in association with elevated blood cortisol. If true, the glucocorticoid receptor could be involved in suppressed functions of mononuclear leukocytes in parturient cows. The objectives of this study were to determine effects of parturition on lymphocyte and monocyte glucocorticoid receptor expression and to correlate expression with serum cortisol concentrations. Objectives were achieved by using fluorescence staining and flow cytometric analyses to monitor glucocorticoid receptors in peripheral blood mononuclear leukocytes collected multiple times from 13 periparturtient test cows (eight multi- and five primiparous) and 10 midgestation control cows (five multi- and five primiparous). Serum cortisol concentrations were determined by radioimmunoassay. Based on intensity of the fluorescent glucocorticoid receptor stain, parturition caused 42 and 47% reductions in lymphocyte and monocyte glucocorticoid receptor expression, respectively, compared with mean expression in corresponding cells from control cows. When mean prepartum values were compared with nadir values at parturition in the test cows, glucocorticoid receptor expression was reduced by 67% in lymphocytes and by 54% in monocytes. Mononuclear cell expression of glucocorticoid receptors was negatively correlated with serum cortisol concentrations. Results suggest that glucocorticoid receptors are down-regulated in bovine mononuclear leukocytes in association with increased adrenal secretion of cortisol at calving. It is possible that glucocorticoid receptor down-regulation is also associated with altered phenotype or function (or both) of lymphocytes and monocytes. This possibility should be substantiated because it could explain increased disease susceptibility in periparturient dairy cows.
(**Key words:** glucocorticoid receptor, leukocytes, periparturition, cattle)

**Abbreviation key: FITC** = fluorescein isothiocyanate, **GC** = glucocorticoid hormones, **GR** = glucocorticoid receptor, **LSM** = least squares means, **MFI** = mean fluorescence density, **PE** = phycoerythrin, **RIA** = radioimmunoassay.

## INTRODUCTION

Dairy cows are susceptible to immunosuppression and mastitis during the peripartum period, a time when blood concentrations of the stress hormone cortisol are high (13, 15, 39). Cortisol is an endogenous glucocorticoid (**GC**) that delivers its hormonal message to cells via cytoplasmic glucocorticoid receptors (**GR**). When bound to GC, GR become activated, translocate into the nucleus, bind to regulatory regions in DNA of glucocorticoid responsive genes, and repress or enhance expression of those genes (1, 2, 8, 25, 26). Therefore, glucocorticoids are critical contributors to altered phenotypes and functions of target cells during stress.

Recent research from our laboratory demonstrated that GR expression is down-regulated in blood neutrophils of periparturient dairy cows in conjunction with elevated blood cortisol concentrations (30). Homologous GR down-regulation has been well documented in a variety of GC responsive systems (2, 26) and presumably provides a short-loop feedback mechanism to protect cells against prolonged exposure to GC (8). Along with homologous GR down-regulation, GC responsive systems also alter expression of other GC responsive

Received February 26, 1999.
Accepted July 19, 1999.
Corresponding author: J. L. Burton; e-mail: burtonj@piolot.msu.edu

genes when GR is activated, thus changing the phenotype and function of the target cells. Down-regulation of the CD62L adhesion molecule is an example of a phenotypic change in response to GC that has been documented in blood neutrophils from both periparturient dairy cows (19) and dairy cattle injected with the potent synthetic GC, dexamethasone (5). Glucocorticoid-induced CD62L down-regulation is associated with dysfunctional neutrophil trafficking that results in neutrophilia and increased mastitis susceptibility (3). Indeed, mastitis susceptibility and neutrophil dysfunctions related to altered trafficking of these leukocytes are well documented for both periparturient and dexamethasone-treated dairy cows (6, 16, 21, 23, 28, 32, 43). Therefore, GR is strongly implicated in neutrophil dysfunctions and mastitis susceptibility of GC-challenged dairy cows.

Mononuclear leukocyte phenotypes and functions of periparturient cows (9, 17, 18, 22, 40) and dexamethasone-treated cattle (4, 24, 33) are also altered. For example, when leukocytes are collected from cows with elevated blood GC, mononuclear cell expression of MHC I and II antigens is down-regulated. Also, trafficking patterns of monocytes and $\gamma\delta$ T cells are altered in vivo, and in vitro proliferation, cytokine, and antibody production in response to mitogens and antigens are inhibited. Each of these cellular phenotypic changes may jeopardize adaptive immunity and suggest that GR could be involved in suppressed immunity and disease susceptibility of GC-challenged cows.

The hypothesis of the present study was that parturition affects bovine mononuclear leukocyte expression of GR and that this is associated with elevated blood cortisol concentrations at calving. The objectives were to monitor GR expression in mononuclear cells throughout the peripartum period and to correlate GR expression with blood cortisol concentration and circulating numbers of lymphocytes and monocytes. Given that homologous down-regulation of GR is caused by GR activation, demonstration of altered GR expression in mononuclear cells in association with elevated blood cortisol levels would circumstantially link GR activation with immunosuppression in periparturient dairy cows.

## MATERIALS AND METHODS

### Cows and Blood Collections

Eight multiparous and five primiparous Holstein cows were used as periparturient test animals, and five multiparous and five primiparous midgestation Holsteins as nonperiparturient control animals. All animals were housed at the Michigan State University (MSU) Dairy Teaching and Research facility and were managed by the facility's herdsmen. Use of animals for this study was approved by the MSU All University Committee for Animal Use and Care.

Blood samples for flow cytometric analysis of lymphocyte and monocyte GR expression and leukocyte counting were collected by tail venipuncture into Vacutainer blood collection tubes containing 1.0 ml of acid citrate dextrose anticoagulant (Becton Dickinson Immunocytometry Systems, Mansfield, MA) with 20 g 2.5-cm multisample needles. Samples for serum harvesting (cortisol assay) were collected into Vacutainer tubes that contained no anticoagulant.

The original schedule for blood collections from periparturient test cows included 11 samples relative to expected calving dates, on d –28, –21, –14, –7, h 0, 12, 24, 20, 48, and d 7 and 14. Pre- and postpartum daily samples were collected at 0800 h, while the intense sampling around calving occurred at precise hours relative to the 0-h (calving) sample. Although five cows calved precisely on schedule, the remaining eight calved earlier than expected. In these cases, prepartum days relative to calving were assigned retrospectively and labeled according to closest day (–21 or –14 d) in the original sampling schedule. In the end, we had data for all 13 test cows from –14 d on, and we had data for seven test cows for –21 d and five cows for –28 d. Therefore, peripartum data more accurately reflected week relative to calving for these cows. Calving dates were also staggered over a 2-mo period (October to December, 1997); multiparous cows calved first, followed by primiparous cows. To insure that adequate control samples were obtained to account for parity and season effects and for assay errors over time, blood samples were collected from at least one parity-matched control cow every time a test animal was sampled. Therefore, there were more total observations for control cows (n = 141) than for test cows (n = 129) in any data set of this study.

### Leukocyte Counting and Standardization of Cell Numbers for GR Staining

Total leukocytes were counted to standardize the number of cells added per well of 96-well U-bottom microtiter plates for fluorescent GR staining, and for subsequent use in determining mononuclear leukocyte counts. Red blood cells from 5 ml of whole blood were lysed for 1.5 min by using 10 ml of cold hypotonic lysing solution (10.56 m*M* $Na_2HPO_4$, 2.67 m*M* $NaH_2PO_4$, pH 7.3). This procedure was followed by the addition of 20 ml of cold hypertonic restoring solution (10.56 m*M* $Na_2HPO_4$, 2.67 m*M* $NaH_2PO_4$, 0.43 M NaCl, pH 7.3). Remaining leukocytes were centrifuged at $1000 \times g$ for 5 min at 22°C, and the supernatants were aspirated.

The cell pellet was washed twice in 2 ml of cold PBS and centrifuged at 800 × *g* for 5 min at 22°C. Leukocytes were suspended in 5 ml of PBS, counted on a hemacytometer, adjusted to $5 \times 10^6$ cells per well, and subjected to fluorescent staining.

### Fluorescent Staining of Mononuclear Leukocyte GR

Prepared leukocytes were differentiated by plasma membrane expression of CD45, and GR was monitored in CD45-differentiated mononuclear leukocytes following fluorescent labeling. The CD45 is a signal transduction molecule that is required for lymphocyte activation (42). It is a transmembrane protein that has different expression levels in various leukocyte populations (highest on lymphocytes, intermediate on monocytes, and lowest on neutrophils) and, therefore, is used as a leukocyte differential in fluorescence activated flow cytometry (41, 42). In the present study, leukocyte CD45 was immunolabeled with a primary antibody (clone CACTB51A, $IgG_{2a}$; VMRD, Pullman, WA) and visualized with a phycoerythrin (**PE**)-conjugated anti-$IgG_{2a}$ secondary antibody (M32204; Caltag Laboratories, Burlingame, CA). Therefore, lymphocytes were differentiated from monocytes in this study by the degree of PE fluorescence intensity. Labeled dexamethasone is commonly used to identify GR in target cells (12, 34) because of its specificity and high affinity for GR. Fluorescein isothiocyanate (**FITC**)-conjugated dexamethasone (Molecular Probes, Eugene, OR) was used at $1 \times 10^{-5}$ *M* in the current study because this concentration saturated the GR of $5 \times 10^6$ cells per well in preliminary studies (data not shown). Specificity of GR labeling was demonstrated when 100- and 500-fold molar excesses of the glucocorticoid antagonists, progesterone and RU486 (both from Sigma Chemical Co., St. Louis, MO) successfully competed with FITC-dexamethasone in this assay (not shown). The dual-color CD45/GR staining protocol was described in detail by Preisler et al. (30).

### Flow Cytometric Acquisition of Mononuclear Leukocyte GR Expression Data

A FACSCalibur fluorescence activated flow cytometer and the acquisition package of CellQuest software (both from Becton Dickinson Immunocytometry Systems) were utilized to acquire lymphocyte and monocyte GR expression data. First, unstained cells were used to check and adjust the forward scatter (FSC = size) and side scatter (SSC = granularity) characteristics of leukocytes in FSC-SSC density plots (Figure la). Next, single-color stained cells were used to set FL-1 (FITC) and FL-2 (PE) detectors and compensation for the two-color analyses. The FL-1, FL-2, and compensation settings were not altered during the actual trial. The FITC and PE negative control cells were used to set background fluorescence. Finally, data from the dual-labeled cells were collected as the lymphocyte and monocyte GR expression data sets used for subsequent hypothesis testing. Data from 5000 leukocytes per sample per cow were acquired.

### Flow Cytometric Analysis of Mononuclear Leukocyte GR Expression Data

The analysis package of CellQuest software (Becton Dickinson) was used to determine lymphocyte and monocyte GR expression, which was recorded as FITC mean fluorescence intensity (**MFI**) of CD45-differentiated leukocytes. Mononuclear leukocytes were differentiated into lymphocytes and monocytes with both their granularity and CD45 staining characteristics (Figure 1b). Glucocorticoid receptor expression was then assessed from FITC fluorescence histograms of the differentiated leukocytes (Figure 1c). Periparturient changes in mononuclear GR expression were observed as shifts in MFI along the X-axis FITC histograms over time, as is demonstrated in Figure 2 for changes in lymphocyte GR expression around calving for one representative test cow. Therefore, lymphocyte and monocyte FITC MFI values were the data sets used to assess effects of parturition on GR expression (see below).

### Leukocyte Counts

Lymphocyte and monocyte counts (number of cells per ml of blood) were determined by multiplying total leukocyte counts (number of leukocytes per ml of PBS; determined by hemacytometer) by the percentage of CD45-differentiated lymphocytes and monocytes in a sample (determined by flow cytometry).

### Blood Cortisol Concentrations

Cortisol concentrations were determined by radioimmunoassay (**RIA**) by using a commercial Coat-A-Count Cortisol Determination Kit (Diagnostics Products Corp., Los Angeles, CA). Sera were harvested from each blood sample following blood clotting and centrifugation of tubes at 1000 × *g* and were stored at –20°C until use in the RIA. All sera were assayed in duplicate on 1 d and data were recorded as counts per minute with a Gamma Trac 1290 (TM Analytic, Elk Grove, IL). The counts per minute were then converted to micrograms of cortisol per deciliter of serum (μg/dl) based on a standard curve of counts per minute by concentration. The RIA intraassay coefficient of variation was 0.65%.

### Statistical Analyses

The SAS PROC MIXED was used to analyze data sets by mixed model analysis (36). The mixed model applied to all data sets included a random cow effect and fixed effects of parity, time (relative to calving), treatment group (control vs. test), calendar week of sampling (accounted for seasonal and assay variation over time), and a fixed time-by-parity interaction, as described in detail by Preisler et al. (30). Because of

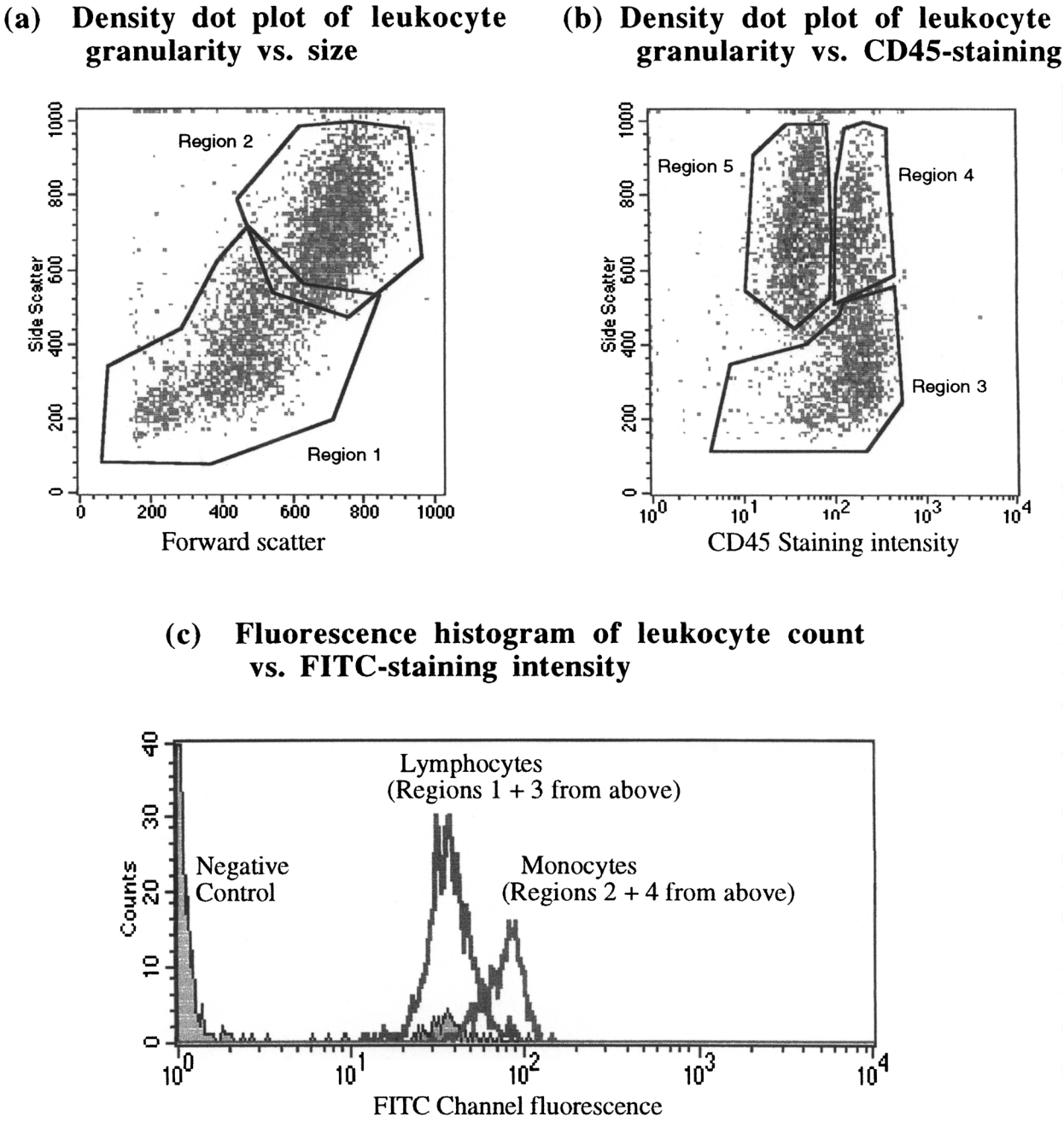


**Figure 1.** Leukocyte differentials were performed by flow cytometry with a combination of cellular granularity and size (a), and cellular granularity and CD45 staining intensity (b). Leukocytes that gated within regions 1 + 3 were identified as lymphocytes, within regions 2 + 4 as monocytes, and within regions 2 + 5 as neutrophils. Mean fluorescein isothiocyanate (FITC) staining intensity of doubly gated lymphocytes and monocytes were used to determine glucocorticoid receptor (GR) expression (c), which was recorded as the geometric mean channel fluorescence (or mean fluorescence intensity, MFI) of each histogram peak. Representative FITC histograms for one control animal show that circulating lymphocytes (MFI = 35.60) express approximately half as much GR as monocytes (MFI = 73.09).

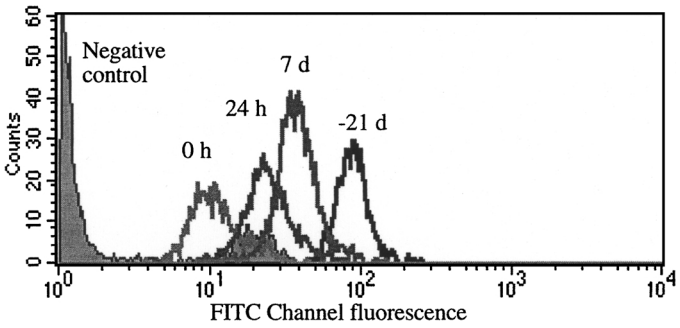


**Figure 2.** Parturition-induced glucocorticoid receptor (GR) down-regulation was determined by shifts in the geometric mean channel fluorescence (MFI) or fluorescein isothiocyanate (FITC)-dexamethasone-stained mononuclear leukocytes. Representative FITC histograms (open peaks) for lymphocytes of one test animal are shown for the following sample times: 21 d precalving (–21 d), calving (0 h), 24 h postcalving (24 h), and 7 d postcalving (7 d). Background fluorescence (negative control) is shown in the solid histogram.

the design of the study, other two-way interactions were confounded and were not included in the model. Upon initial examination of the distribution of GR expression data sets, log transformations were applied to MFI values. Cortisol and leukocyte count data sets were analyzed without transformation. Time and time-by-parity least squares means (**LSM**) and standard errors of the means are reported for lymphocyte and monocyte GR expression (log MFI), cortisol (micrograms per deciliter), and leukocyte, lymphocyte, and monocyte counts (number of cells per milliliter). The correlation procedure of SAS (35) was used to determine associations between lymphocyte and monocyte GR expression and blood cortisol concentrations, leukocyte, lymphocyte, and monocyte counts. All available data from control and test cows are reported. Statistical significance was considered at $P \leq 0.05$.

## RESULTS

### GR Expression in Bovine Mononuclear Leukocytes

Fluorescence staining of lymphocytes and monocytes indicated that, on average, monocytes had approximately twice the GR expression of lymphocytes (Figure lc). The MFI values represented the geometric mean of the FITC intensity peaks for each leukocyte type (as indicated by the mean channel fluorescence on the X-axis in Figure 1c) contained in 5000 total leukocytes counted from each blood sample. Studies in humans also demonstrated twice as many GR molecules in monocytes as in lymphocytes (10, 20, 37, 38).

### GR Expression in Lymphocytes of Periparturient Cows

Fluorescence histograms from multiple periparturient blood samples for one test animal (Figure 2) demonstrate shifts to the left of the 0- (calving) and the 24-h histograms relative to the precalving (–21 d) histogram, as well as incomplete recovery of the GR expression peak by 7 d postcalving. Parturition induced dramatic and chronic GR down-regulation in circulating lymphocytes from this animal. Similarly, MFI of all lymphocytes from periparturient test animals at calving were lower than precalving MFI. Analysis of variance of lymphocyte GR expression revealed significant effects of time, calendar week, and the parity-by-time interaction (Table 1). As demonstrated by the time LSM in Figure 3a, parturition caused a significant reduction ($P < 0.0001$) in FITC-dexamethasone staining in lymphocytes, which started during the week prior to calving. In Figure 3, time relative to parturition on the X-axis is reported in days to provide accuracy in data spacing over time, therefore, 12 h equals 0.5 d. When converted back to the original unit of measurement (MFI), lymphocytes from test animals at calving had a mean 42% reduction ($P < 0.0001$) in GR expression relative to the mean GR expression in lymphocytes from control animals. The reduction in lymphocyte GR expression persisted to 24 h ($P < 0.0001$) and remained significantly lower ($P < 0.05$) than control GR values throughout the remaining 14-d postcalving period (Figure 3a). When compared with precalving values (mean of –28, –21, –14, and –7 d) of the test animals themselves, GR expression was reduced by 67% at calving.

### GR Expression in Monocytes of Periparturient Cows

Analysis of variance of monocyte GR expression revealed significant effects of time, calendar week, and the parity-by-time interaction (Table 1). Time LSM in Figure 3b demonstrated that parturition caused a significant reduction ($P < 0.0001$) in monocyte GR expression. By calving, monocyte GR down-regulation (MFI) reached a nadir that was 47% lower ($P < 0.0001$) than GR expression in monocytes of control animals and 54% lower ($P < 0.0001$) than the precalving mean (–28, –21, –14, and –7 d) GR expression in the test cows. Significant ($P < 0.0001$) down-regulation of monocyte GR expression persisted to 24 h postcalving and remained lower ($P < 0.05$) than control GR values throughout the remaining 14 d of the study.

### Blood Cortisol Concentrations

The main effect of time relative to parturition was significant ($P < 0.0001$; Table 1) for blood cortisol con-

**Table 1.** *P* values for terms in the mixed model analysis of variance of mononuclear leukocyte glucocorticoid receptor (GR) expression, serum cortisol concentration, and total counts of lymphocytes and monocytes.

| | Mononuclear leukocyte GR expression | | | Mononuclear leukocyte counts | |
|---|---|---|---|---|---|
| Model effect | Monocytes (log of MFI[1]) | Lymphocytes (log of MFI) | Cortisol concentration ($\mu$g/dl) | Lymphocytes (# cells/ml) | Monocytes (# cells/ml) |
| Time | 0.0001 | 0.0001 | 0.0001 | 0.5309 | 0.0099 |
| Parity | 0.3788 | 0.2894 | 0.1571 | 0.7235 | 0.0091 |
| Calendar wk | 0.0046 | 0.0001 | 0.7174 | 0.0001 | 0.0059 |
| Parity × time | 0.0023 | 0.0036 | 0.1435 | 0.3075 | 0.6196 |

[1]MFI = mean fluorescence intensity.

centrations. Time LSM for cortisol were increased nearly sevenfold at calving (0 h) compared to control and precalving means (Figure 3c), but the elevation was short-lived and returned to basal concentrations by 24 h postcalving. Cortisol concentration was negatively correlated with lymphocyte GR expression (r = –0.213; $P < 0.05$) and monocyte GR expression (r = –0.43; $P < 0.05$) overall.

## Parity-by-Time Interaction Effects on Mononuclear Leukocyte GR Expression

The parity-by-time interaction effect was significant (Table 1) for the lymphocyte GR ($P < 0.004$; Figure 4a)

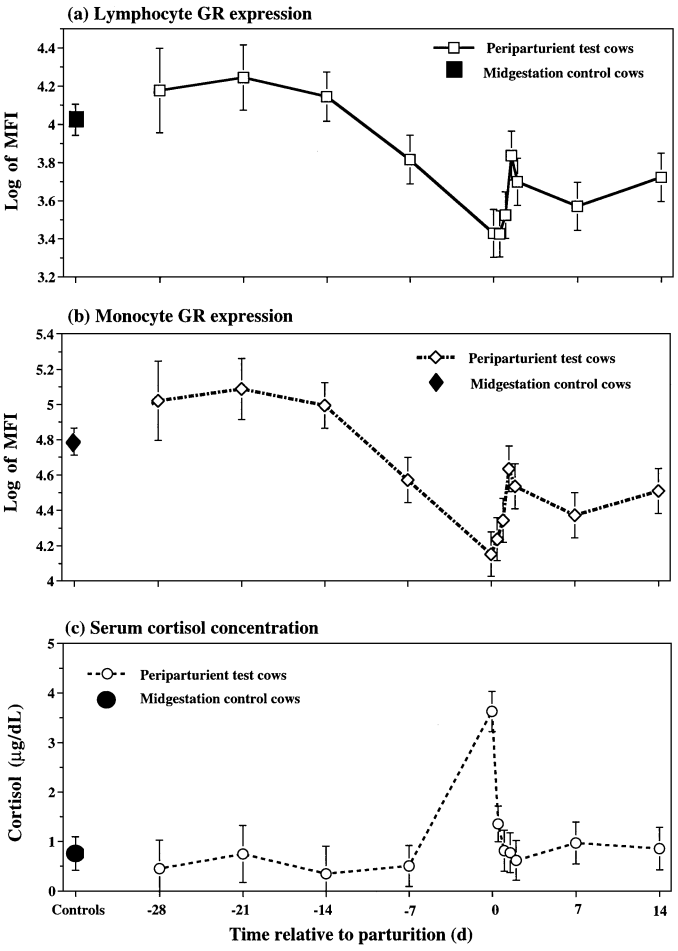


**Figure 3.** Time least squares means (± SEM) of lymphocyte glucocorticoid receptor (GR) expression (a), monocyte GR expression (b), and serum cortisol concentrations (c) in periparturient test cows (open symbols) and midgestation control cows (solid symbols). Parturition occurred at time 0 h.

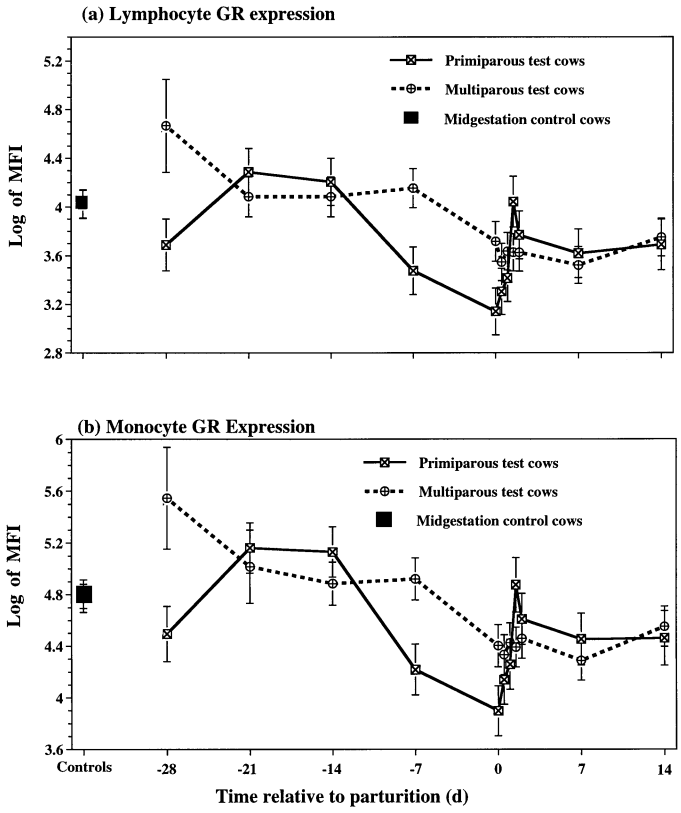


**Figure 4.** Parity-by-time least squares means (± SEM) of lymphocyte (a) and monocyte (b) glucocorticoid receptor (GR) expression in primiparous test cows (x'ed squares), multiparous test cows (x'ed circles), and midgestion control cows (solid symbols). Parturition occurred at time 0 h.

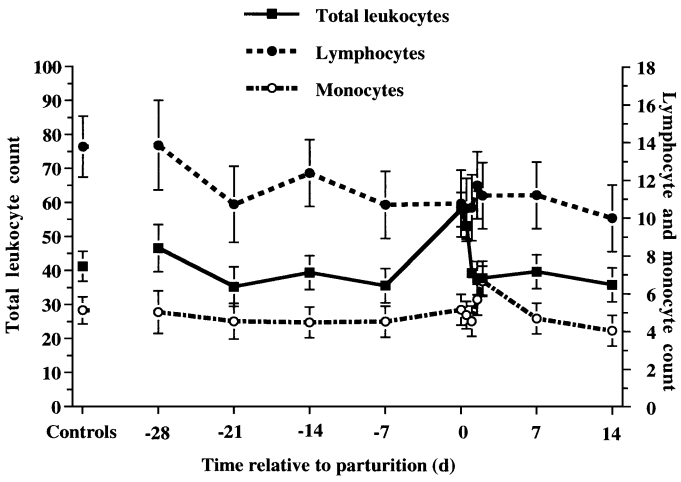


**Figure 5.** Time least squares means (± SEM) for total leukocyte (solid squares), lymphocyte (solid circles), and monocyte counts (open circles) in periparturient test cows (lines) and midgestation control cows (single symbols). Parturition occurred at time 0 h.

and monocyte GR ($P < 0.003$; Figure 4b) expression data sets. Parity-by-time LSM in Figure 4 demonstrated that mononuclear leukocytes from primiparous cows had earlier, more acute reductions in GR expression, followed by more rapid increases in expression to 36 h postcalving than leukocytes from multiparous cows. Glucocorticoid receptor down-regulation was more gradual and persistent in the multiparous cows.

### Lymphocyte and Monocyte Counts

Significant time relative to parturition effects were observed for monocyte counts ($P < 0.01$) but not for lymphocyte counts ($P > 0.53$) (Table 1). Time LSM in Figure 5 demonstrated that parturition increased the total number of circulating leukocytes, which was associated with neutrophilia at that time (30). Parturition did not alter the number of circulating lymphocytes and only modest elevations in monocyte counts were observed 48 h postcalving ($P < 0.03$). In this study, the main effect of calendar week was significant for lymphocyte and monocyte counts ($P < 0.001$, $P < 0.006$; Table 1). There was also a significant parity effect ($P < 0.01$) on monocyte counts that resulted in multiparous test cows having higher overall circulating monocyte counts ($5.2 \times 10^6$ cells/ml) on average that primiparous test cows ($3 \times 10^6$ cells/ml). No correlations existed ($P > 0.10$) between GR expression or cortisol concentration and mononuclear leukocyte counts.

## DISCUSSION

It is difficult to manage dairy cows during the periparturn period because of parturition-associated increases in metabolic disorders and infectious diseases, including mastitis. The natural stress of calving normally occurs several times in the productive lifetime of a particular cow, and with each parity the incidence and severity of disorders increases (7, 14). Also, parturition is associated with dysfunction of most leukocytes including altered expression of key proteins involved in trafficking, migration, antigen presentation, and activation (4, 5, 9, 16, 17, 18, 19, 24). The exact mechanisms by which leukocyte functions are altered around parturition are unknown. The findings of this study provide preliminary data that begin to support a potential role for GR regulation in periparturient immunosuppression related to leukocyte dysfunction and disease susceptibility. The observed reductions in lymphocyte and monocyte GR expression at calving in this study, and negative correlations between mononuclear cell GR expression and blood cortisol concentration, suggest that cortisol-activated GR might be involved in altered gene transcription in these cells around parturition.

Glucocorticoid receptor down-regulation observed for bovine mononuclear leukocytes in this study started in the week prior to calving. As demonstrated in Figure 4a and b, most of this decline in GR was attributed to mononuclear leukocytes of the primiparous cows and occurred slightly before increases in blood cortisol were detectable (Figure 3). Therefore, it is possible that other reproductive or nutrient partitioning hormones (or both) can precipitate GR down regulation in bovine leukocytes of primiparous cows. Although this possibility requires substantiation, reductions in mean FITC MFI on –7 d relative to –28, –21, and –14 d for all cows were modest and were not statistically different from the mean values for control cows. Therefore, virtually all of the detected "parturition effects" (i.e., parturient vs. control cows) on mononuclear leukocyte GR expression began at the 0-h sample in this study, most likely in association with the sevenfold increase in blood cortisol.

Literature on GR regulation in species other than cattle offers several explanations for the parturition-induced GR down-regulation observed in the present study. Receptor degradation following GC activation of GR partly explains homologous down-regulation of the receptor protein in many human cells, including lymphocytes (2, 26). If degradation of the GR protein occurred in response to elevated cortisol in the current study, fewer GR molecules would have been available to bind with FITC-dexamethasone and could have resulted in the reduced FITC MFI values. In fact, it appears that homologous GR down-regulation occurs at many levels in all GC-sensitive tissues studied so far in humans and rodents (11, 37, 38). Therefore, it is not unreasonable to speculate that the GR down-regulation observed bovine mononuclear cells in this study was

also caused by homologous down-regulation in response to cortisol secretion around calving. In addition to GR protein degradation, literature on humans cites clear evidence that the rate of GR gene transcription is reduced by GC-activated GR, leading to 30 to 80% reductions in GR mRNA abundance that readily explain reductions in receptor protein abundance (2, 27, 29, 31). The possibility that this also occurs in mononuclear leukocytes of periparturient cows is currently being explored in our laboratory to explain the observed reductions in FITC-dexamethasone binding, presumably indicating homologous GR down-regulation. All of these possibilities have implications for GR being involved in both its own autoregulation and the transcriptional regulation of other molecules in bovine lymphocytes and monocytes that are critical for effective immune responses. These possibilities should be explored in periparturient dairy cows as a means of explaining disease susceptibility around calving.

Studies with periparturient dairy cows and cattle treated with dexamethasone have documented that leukocyte functions and phenotypes are dramatically altered when blood GC concentrations are high (5, 16, 17, 18). For example, parturition impairs neutrophil phagocytic functions (6, 23, 32) and expression of the CD62L adhesion molecule (19), both of which facilitate important protective roles of neutrophils when the mammary gland becomes infected with mastitis-causing bacteria. It is not surprising then, that the periparturient period is one of high mastitis susceptibility (28). Decreased neutrophil CD62L expression and increased mastitis susceptibility are also observed when cattle are injected with dexamethasone (3, 5). Indeed, mastitis susceptibility is a general phenomenon of dexamethasone-treated cows (21, 43). Dexamethasone also has profound effects on mononuclear cells in cattle, including inhibition of IFN-$\gamma$ and IgM secretion (24) and decreased expression of MHC I and MHC II molecules (4). Because dexamethasone binds with high affinity to GR, these alterations in key leukocyte functions and phenotypes imply that GR is involved in dysfunctions of the bovine immune system during periods of GC challenge. Our mononuclear leukocyte GR expression and blood cortisol data suggest that parturition is one of these periods in dairy cows.

Parity differences in this study showed that multiparous animals had gradual but prolonged lymphocyte and monocyte GR down-regulation when compared with primiparous animals. Primiparous cows did not develop metabolic disorders or mastitis, whereas more than half of the multiparous cows did form some combination of these disorders. This outcome was not surprising because multiparous cows have higher incidences of health problems associated with parturition than do primiparous cows (7, 14). Because of the small population in this study, statistical analyses of health effects on leukocyte GR expression were not attempted. However, exaggerated persistency of GR down-regulation in multiparous test cows could indicate that mononuclear leukocytes of these animals are more sensitive to parturition-related increases in blood cortisol than cells from primiparous animals. If GR regulation in peripheral tissues, including leukocytes, is related to stress adaptation and leukocyte dysfunction this could facilitate disease susceptibility. On the other hand, the stress of overcoming parturition in conjunction with the presence of metabolic disorders could have resulted in prolonged reduction of lymphocyte and monocyte GR expression in multiparous cows. Another related possibility is that higher milk production demands of multiparous versus primiparous cows could have stressed homeostasis to a larger degree, resulting in exaggerated GR down-regulation, even in the face of normal cortisol concentrations beyond 24 h into lactation. Certainly, the effects of other reproductive and nutrient partitioning hormones, and of disease on leukocyte GR expression, function, and phenotype warrants further investigation. In any case, the degree of lymphocyte and monocyte GR down-regulation following parturition may be a better indicator of stress than blood cortisol concentrations and offers a possible explanation for the higher incidence of metabolic disorders and mastitis susceptibility in multiparous versus primiparous periparturient dairy cows. These possibilities require substantiation through experiments designed specifically to assess the role of GR down-regulation in gene expression (phenotype) and functional capacity of leukocytes, and in peripheral tissue sensitivity to homeostatic and homeorhetic hormones in periparturient dairy cows.

## CONCLUSIONS

The present study demonstrated glucocorticoid receptor down-regulation in bovine peripheral blood lymphocytes and monocytes at parturition in association with elevated maternal blood cortisol concentrations. Observed glucocorticoid receptor down-regulation has potential implications regarding possible mechanisms regulating immunocompetence and disease susceptibility around parturition, especially in multiparous cows.

## ACKNOWLEDGMENTS

The authors would like to thank Doug Mashek for assistance during sample collections, Jennifer Wells and Tracy Davis for laboratory assistance, Yan Yuan for assistance during RIA of serum cortisol, Peter

Saama for statistical assistance, and Bob Kreft and the MSU Dairy Teaching and Research Farm staff for animal care. This work was funded through the Michigan Agricultural Experiment Station, project number MICL01836.

## REFERENCES


1 Bamberger, C. M., H. M. Schulte, and G. P. Chrousos. 1996. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr. Rev. 17:245–259.

2 Burnstein, K. L., and J. A. Cidlowski. 1992. The down side of glucocorticoid receptor regulation. Mol. Cell. Endocrinol. 83:Cl C8.

3 Burton, J. L., and M. E. Kehrli, Jr. 1995. Regulation of neutrophil adhesion molecules and shedding of *Staphylococcus aureus* in milk of cortisol- and dexamethasone-treated cows. Am. J. Vet. Res. 56:997–1006.

4 Burton, J. L., and M. E. Kehrli, Jr. 1996. Effects of dexamethasone on bovine circulating T lymphocyte populations. J. Leukoc. Biol. 59:90–99.

5 Burton, J. L., M. E. Kehrli, Jr., S. Kapil, and R. L. Horst. 1995. Regulation of L-selection and CD 18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. J. Leukoc. Biol. 57:317–325.

6 Cai, T-Q., P. G. Weston, L. A. Lund, B. Brodie, D. J. McKenna, and W. C. Wagner. 1994. Association between neutrophil functions and periparturient disorders in cows. Am. J. Vet. Res. 55:934–943.

7 Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J. Dairy Sci. 68:2347–2360.

8 DeRijk, R., and F. Berkenbosch. 1994. Suppressive and permissive actions of glucocorticoids: A way to control innate immunity and to facilitate specificity of adaptive immunity? Pages 73–95 *in* Endocrinology and Metabolism # 7; Bilateral Communication Between the Endocrine and Immune Systems. C. J. Grossman, ed. Springer-Verlag, Inc., New York, NY.

9 Detilleux, J. C., M. E. Kehrli, Jr., J. R. Stabel, A. E. Freeman, and D. H. Kelley. 1995. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. Vet. Immunol. Immunopathol. 44:251–267.

10 Distelhorst, C. W., and B. M. Benutto. 1981. Glucocorticoid receptor content of T lymphocytes: Evidence for heterogeneity. J. Immunol. 126:1630–1634.

11 Dong, Y., L. Poellinger, J-A. Gustafson, and S. Okret. 1988. Regulation of glucocorticoid receptor expression: Evidence for transcriptional and posttranscriptional mechanisms. Mol. Endocrinol. 2:1256–1264.

12 Gagne, D., M. Pons, and A. C. De Paulet. 1986. Analysis of the relation between receptor binding affinity and antagonist efficacy of antiglucocorticoids. J. Steroid Biochem. 25:315–322.

13 Goff, J. P., M. E. Kehrli, Jr, and R. L. Horst. 1989. Periparturient hypocalcemia in cows: prevention using intramuscular parathyroid hormone. J. Dairy Sci. 72:1182–1187.

14 Grohn, Y. T., S. W. Eicker, and J. A. Hertl. 1995. The association between previous 305-day milk yield and disease in New York State dairy cows. J. Dairy Sci. 78:1693–1702.

15 Guidry, A. J., M. J. Paape, and R. E. Pearson. 1976. Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids, and neutrophil phagocytosis in the cow. Am. J. Vet. Res. 37:1195–1200.

16 Kehrli, M. E., J. L. Burton, B. J. Nonnecke, and E. K. Lee. 1998. Effects of stress on leukocyte trafficking and immune responses: Implications for vaccination. Pages 61–81 *in* Advances in Veterinary Medicine, Vol 41. R. D. Schultz, ed. Academic Press, New York, NY.

17 Kehrli, M. E., Jr, B. J. Nonnecke, and J. A. Roth. 1989. Alterations in bovine neutrophil function during the periparturient period. Am. J. Vet. Res. 50:207–214.

18 Kehrli, M. E., Jr, B. J. Nonnecke, and J. A. Roth. 1989. Alterations in bovine lymphocyte function during the periparturient period. Am. J. Vet. Res. 50:215–220.

19 Lee, E-K., and M. E. Kehrli, Jr. 1998. Expression of adhesion molecules on neutrophils of periparturient cows and neonatal calves. Am. J. Vet. Res. 59:37–43.

20 Lipman, M., and R. Barr. 1977. Glucocorticoid receptors in purified subpopulation of human peripheral blood lymphocytes. J. Immunol. 118:1977–1981.

21 Lohuis, J.A.C.M., W. Van Leeuwen, J.H.M. Verheijden, A.S.J.-P.A.M. Van Miert, and A. Brand. 1988. Effect of dexamethasone on experimental *Escherichia coli* mastitis in the cow. J. Dairy Sci. 71:2782–2789.

22 Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. Lacey Vankampen, L. Wagter, and B. N. Wilkie. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. J. Dairy Sci. 81:585–595.

23 Nagahata, H., S. Makino, H. Takahashi, and H. Noda. 1988. Assessment of neutrophil function in the dairy cow during the perinatal period. J. Vet. Med. 35:747–751.

24 Nonnecke, B. J., J. L. Burton, and M. E. Kehrli. 1997. Associations between function and composition of blood mononuclear leukocyte populations from Holstein bulls treated with dexamethasone. J. Dairy Sci. 80:2403–2410.

25 Norman, A. W., and G. Litwack. 1997. Hormones. Second Edition. Academic Press, California.

26 Oakley, R. H., and J. A. Cidlowski. 1993. Homologous down regulation of the glucocorticoid receptor: The molecular machinery. CRC Crit. Rev. Eukaryot. Gene Expression 3:63–88.

27 Okret, S., L. Poellinger, Y. Dong, and J-A. Gustafson. 1986. Down-regulation of glucocorticoid receptor mRNA by glucocorticoid hormones and recognition by the receptor of a specific binding sequence within a receptor cDNA clone. Proc. Natl. Acad. Sci. USA 83:5899–5903.

28 Oliver, S. P., and L. M. Sordillo. 1988. Udder health in the periparturient period. J. Dairy Sci. 71:2584–2606.

29 Petrichenko, I. E., Y. A. Shakhov, N. A. Gratsianski, O. I. Aleshin, N. V. Chepurnenko, and N. V. Perova. 1989. Number of glucocorticoid receptors in lymphocytes and their sensitivity to hormone action. Experientia 45:1115–1117.

30 Preisler, M. T., P.S.D. Weber, R. J. Tempelman, R. J. Erskine, H. Hunt, and J. L. Burton. Glucocorticoid receptor down-regulation in neutrophils of periparturient cows. Am. J. Vet. Res. (in press).

31 Rosewicz S., A. R. McDonald, B. A. Maddux, I. D.Goldfine, R. L. Miesfeld, and C. D. Logsdon. 1988. Mechanism of glucocorticoid receptor down-regulation by glucocorticoids. J. Biol. Chem. 263:2581–2584.

32 Roth, J. A., and M. L. Kaeberle. 1981. Effects of in vivo dexamethasone administration on in vitro bovine polymorphonuclear leukocyte function. Infect. Immunol. 33:434–441.

33 Roth, J. A., and M. L. Kaeberle. 1982. Effect of glucocorticoids on the bovine immune system. JAVMA 180:894–901.

34 Rupprecht, R., J.M.H.M. Reul, B. van Steensel, D. Spengler, M. Soder, B. Berning, F. Holsboer, and K. Damm. 1993. Pharmacological and functional characterization of human mineralcorticoid and glucocorticoid receptor ligands. Eur. J. Pharmacol. 247:145–154.

35 SAS Procedures Guide, Version 6, Third Edition. 1990. SAS Inst., Inc., Cary, NC.

36 SAS/STAT Software: Changes and Enhancement through release 6.11. 1996. SAS Inst., Inc., Cary, NC.

37 Schlecte, J. A., B. H. Ginsberg, and B. M. Sherman. 1982. Regulation of the glucocorticoid receptor in human lymphocytes. J. Steroid Biochem. 16:69–74.

38 Shipman, G. F., C. D. Bloomfield, K. J. Gajil-Peczalska, A. U. Munck, and K. A. Smith. 1983. Glucocorticoids and lymphocytes.

III. Effects of glucocorticoid administration on lymphocyte glucocorticoid receptors. Blood 61:1086–1090.
39 Smith, V. G., L. A. Edgerton, H. D. Hafs, and E. M. Convey. 1973. Bovine serum estrogens, progestins and glucocorticoids during late pregnancy, parturition, and early lactation. J. Anim. Sci. 36:391–396.
40 Sordillo, L. M., M. J. Redmond, M. Campos, L. Warren, and L. A. Babiuk. 1991. Cytokine activity in bovine mammary gland secretions during the periparturient period. Can. J. Vet. Res. 55:298–301.
41 Sutherland, R. D., A. Keating, R. Nayar, S. Anania, and A. K. Stewart. 1994. Sensitive detection and enumeration of $CD34^+$ cells in peripheral cord blood by flow cytometry. Exp. Hematol. 22:1003–1010.
42 Trowbridge, I. S., and M. L. Thomas. 1994. CD45: An emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. Annu. Rev. Immunol. 12:85–116.
43 Wesley, I. V., J. H. Bryner, M. J. Van Der Maaten, and M. Kehrli. 1989. Effects of dexamethasone on shedding of *Listeria monocytogenes* in dairy cattle. Am. J. Vet. Res. 50:2009–2013.